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Original Research Article

Unveiling the Bioactive Power of *Apis Mellifera* (L); Isolates and Their Role In Anti Inflammatory Activity

Biju C. Ramankutty[1] Jyothisree Gangadharan[2] *, Ayswarya Karingali [3], Arunlal V. Balan [4], Anilasree B. Parambath [5], Shitha Gopalakrishnan[6]

[1]*Professor, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala,*
[2]*Professor, Jamia Salafiya Pharmacy College, Pulikkal, Malappuram, Kerala,*
[3]*Associate Professor, Devaki Amma Memorial College of Pharmacy, Malappuram, Kerala,*
[4] *Professor, National College of Pharmacy, Manassery, Kozhikode, Kerala,*
[5]*Professor, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala,*
[6]*Assistant Professor, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, Shitha Gopalakrishna*

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ABSTRACT

Apis mellifera (L.), a rich source of several phytochemical elements, is used to treat a wide variety of illnesses and infections. This study investigated the anti-inflammatory effects of the bioactive and metabolic products of *Apis mellifera* (L.). Seventy percent ethanol was used for continuous Soxhlet extraction of *Apis mellifera*. The different bioactive components included in the extract were isolated via a column chromatographic technique. Various spectroscopic techniques have been used to describe the structure of the isolated molecule. Studies on the effects of single substances on inflammation have been conducted both acutely and chronically. Using a variety of spectroscopic techniques, the molecular weight of the isolated compound was determined to be 866.74, and procyanidin, a trimer of catechine, was identified as the cause. Acute oral toxicity tests revealed that the extract was safe at concentrations up to 2000 mg/kg. Two hundred mg/kg and 400 mg/kg of isolate were selected for assessment of anti-inflammatory activity. Given that the isolate procyanidins had a significant anti-inflammatory effect at 400 mg/kg, these compounds may be responsible for the anti-inflammatory effect on *Apis mellifera* (L.).

Keywords: *Apis mellifera* (L.), bioactive constituents, procyanidin, anti-inflammatory activity.

Introduction

Africa, Western Asia, and Europe are the native habitats of bees (*Apis mellifera* (L.). The introduction of these bees to other continents by humans in the 17th century led to their spread. *Apis mellifera* (L.) has a reddish-brown abdomen with black bands that are rimmed in orange and yellow. They also have a pollen basket on their hind legs. The majority of honey bees have dark brown or black legs. There are two castes of females, namely, fertile queens and sterile workers, which are smaller (10–15 mm) and longer (18–20 mm). Male drones reach a mature length of 15–17 mm. The stingers of both female castes are composed of modified ovipositor parts[1]. Traditional medicine has long employed the venom of honey bees to manage a wide range of inflammatory conditions, including lupus, tendinitis, rheumatism, arthritis, and angia cardiopathy. Additionally, a number of studies have documented its cytotoxic qualities against a range of tumor cell types, including leukemic cancer cells and those from the liver, prostate, breast, and lung. The applicability of bee venom is limited by several drawbacks, including its potent neurotoxicity, cardiotoxicity, and hemolytic and immunogenic effects. Propolis is a complex mixture of chemicals secreted by bees and those taken from plants. Chemically, it is a resin that tastes like vanilla, honey, wax, and well-known buds. However, it may taste bitter. It is either brown or dark green [2-5]The objective of this effort was to separate the active ingredients that influence the anti-inflammatory properties of *Apis mellifera* (L.) Many orthodox anti-inflammatory medications are available, but most of them have long- or short-term adverse effects; thus finding novel agents that are safe for both short- and long-term use is crucial. The goal of this study was to identify and isolate the anti-inflammatory properties of *Apis mellifera* (L.) as well as the bioactive components that has have the greatest therapeutic potential against inflammation. The objective of this study was to evaluate the anti-inflammatory efficacy of metabolic products *via various* in vivo methodologies. Additionally, docking studies were used to assess the possible targets/ interactions of the isolated compounds with the receptor.

*Corresponding author. Email: jyothi.g.marar@gmail.com
Tel: +91 7907662579

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Materials and Methods

Collection and identification of bees

Insect was collected in the month of October 2017 and authenticated by Dr. M. Nasser, Zoologist, Department of Zoology, University of Calicut, Thenhipalam, Kerala, India. The specimen voucher (Number: AM- KZ-2017-03) was deposited in the Department of Zoology, University of Calicut, itself.

Extraction

Fresh *Apis mellifera* (L.) bees were killed and allowed to dry in the shade. Using a Soxhlet apparatus, a continuous hot percolation procedure was used to prepare a sample of hydroalcoholic bee extract for three hours. A thimble containing approximately 900 grams of evenly packed bee material was extracted using 1000 milliliters of hydro alcohol. The extracts were filtered and concentrated at room temperature to obtain a dry mass. [6-8]

Phytochemical identification test
Phytochemical assays to identify the constituents of the hydroalcoholic extract of *Apis mellifera* (L.)[9] were conducted.

Column chromatography
A glass column (50 cm × 2.5 cm) was packed with silica gel (60–120 mesh) using an *n*-butanol slurry to achieve uniform packing and minimize air gaps within the stationary phase. The *Apis mellifera* (L.) extract was dissolved in analytical-grade *n*-butanol to obtain a homogeneous solution and subsequently adsorbed onto a pre-weighed quantity of silica gel (1:2 w/w, extract to silica gel) to ensure even loading. The dried, extract-impregnated silica gel was carefully layered onto the packed column via a glass funnel to avoid disturbance of the stationary phase.
n-Butanol was used as the mobile phase, and the column was allowed to equilibrate for 30 minutes to facilitate proper settling and adsorption of the sample. Elution was performed under gravity at a controlled flow rate of 1–2 mL/min, and fractions of 10 mL were collected sequentially in clean, labelled vials. Each fraction was monitored by thin-layer chromatography (TLC) using silica gel 60 F254 plates with *n*-butanol as the developing solvent. The TLC spots were visualized under UV light (254 nm and 365 nm) and by spraying with detecting reagents (e.g., anisaldehyde- sulfuric acid) to confirm the presence of phytoconstituents.
Fractions exhibiting similar TLC profiles were pooled and concentrated under reduced pressure using a rotary evaporator. The resulting concentrated fractions were subjected to further phytochemical evaluation following standard protocols

Spectral characterization
IR, NMR, and mass spectrometry were used to analyze the isolated substance and ascertain its chemical structure [10].

Pharmacological studies
The IAEC of the institution authorized the animal's study procedure. Its registration number is DAMCOP/IAEC/025.

Acute oral toxicity study
Swiss albino mice were utilized in the acute oral toxicity investigation following the modified procedure set out by OECD No. 425 (OECD 425, 1988). Following 24 hr of fasting, six animals (three for each phase) were employed in an experiment at a dose of 2 g/kg body mass. Following treatment, every animal was observed separately at least once every 30 minutes for the first 24 hours. For the following 14 days, additional attention was given daily, particularly during the first four hours[11].

In vivo examination
Anti-inflammatory investigations utilizing a rat paw edema model with carrageenan
The Wistar rats were separated into four groups of five rats each. In Group 1, the power group received normal saline, whereas in Group 2, the power group received indomethacin (10 mg/kg, p.o. Groups 3 and 4 were given test samples at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively. The control vehicle, reference drug and test samples were administered 30 minutes prior to carrageenan injection. After this interval, 0.1 ml of 1% w/v carrageenan solution prepared in 0.9% normal saline was injected into the plantar area of the right hind paw. The paw volume of each group of rats was assessed at 0, 30, 60, 120, 180, and 240 min following carrageenan challenge[12, 13].

Chronic anti-inflammatory studies with cotton pellets induced inflammatory granulomas
The four groups of Wistar rats, each consisting of five animals, were treated via the cotton pellet-induced inflammatory granuloma method. Thirty minutes prior to the implantation of the cotton pellets, the animals were given the vehicle, the usual medication, or the test medication. Thirty minutes after the initial dosage, a small subcutaneous incision was made in the sedated animals, and 10 ± 0.5 mg of sterile cotton pellet was positioned close to each axilla region. The treatments were continued for seven days. The cotton pellets were surgically removed on the 8th day, and the rats were sacrificed under deep anesthesia. After any extraneous tissue was removed from the pellet, the pellet was weighed. Thereafter, the pellet was dried at 60 °C until the weight stabilized. The increase in weight of the cotton pellets as a percentage of inhibition was computed via the following equation:

$$\%\ inhibition = \left(\frac{Wc-Wd}{Wc}\right) X100$$

- W_c = Weight (or absorbance) of control
- W_d = Weight (or absorbance) of drug/sample

Statistical analysis
The findings are presented as the mean ± standard deviation following analysis via Student's t test. To evaluate differences among groups, one-way analysis of variance (ANOVA) was conducted, followed by the Tukey–Kramer multiple comparison method[14].

Results and Discussion

Extraction
The percentage yield was 33% w/w. Phytochemical analyses of the hydroalcoholic extract of *Apis mellifera* (L.) revealed the presence of phytoconstituents.

Column chromatography
Column chromatography of the hydroalcoholic extract yielded significant fractions in an n-butanol: ethanol (80:20v/v) mobile phase. TLC was used to verify the fractions. A mobile phase composed of n-butanol, acetic acid, and water (4:1:5 v/v/v) and an iodine chamber were used as the detection agent, and the isolated chemical was identified. The acquired R_f value of 0.78 was comparable to the R_f value of flavonoids.

Spectral characterization
Infrared spectroscopy
The structure of *Apis mellifera* (L.) was determined via a Jasco - 4600 Fourier transform infrared (FTIR) spectrophotometer. We measured the IR values in cm^{-1}. The observations revealed four distinct peaks: one at 3100 cm^{-1}, one at 3010 cm^{-1}, one at 1400 cm^{-1}, one at 1231 cm^{-1}, and one at 1400 cm^{-1}, which suggested the presence of aromatic C–H stretching, C–H stretching, and COC (diaryl) groups (Figure 1).

NMR spectroscopy
NMR spectra of the *Apis mellifera* (L.) isolate were obtained via a Bruker Avance III 400 MHz spectrometer from the School of Chemistry, University of Hyderabad, Gachibowli, and Hyderabad. The isolate was dissolved in DMSO, and the values were measured in δ ppm.
After the ^1H NMR spectra of the *Apis mellifera* (L.) isolate were integrated with the following values, it was determined that the existence of a proton linked to the benzene ring was indicated by the peaks at δ 5.66, 5.75, 5.71, and 6.45 ppm. The signal at δ 5.0 ppm indicated that a proton of OH was attached to a benzene ring. A proton connected to the methane group was visible at the peak at δ 5.05 ppm. A proton connected to the CH_2 group was identified by the signal at δ 2.45 ppm. A proton connected to the hexane group was confirmed by the peak at δ 6.45 ppm.
The following values were used to integrate the ^{13}C NMR spectra of the *Apis mellifera* (L.) isolate, providing information about the carbon attached to the isolate's structure. The signal at δ 79.52 ppm indicated that C was attached to the O_2 group. The peak at δ 157.5 ppm suggested that tertiary C was associated with the O_2 group. The peak at δ 158 ppm suggested that the secondary C was connected to the OH group. The peak at δ 79.8 ppm suggested that the C was connected to O in the pyran ring. Secondary carbon was detected by the signal at δ 29.2 ppm. A tertiary C atom was detected by the signal at δ 26.8 ppm.

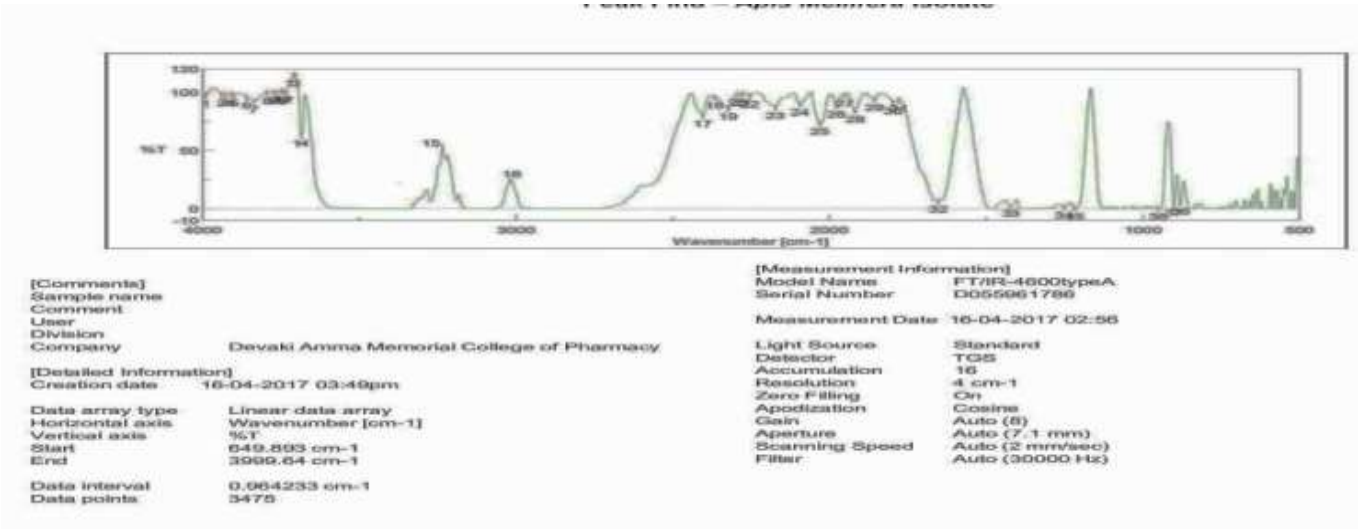

Figure 1: IR spectrum analysis of the isolated compounds

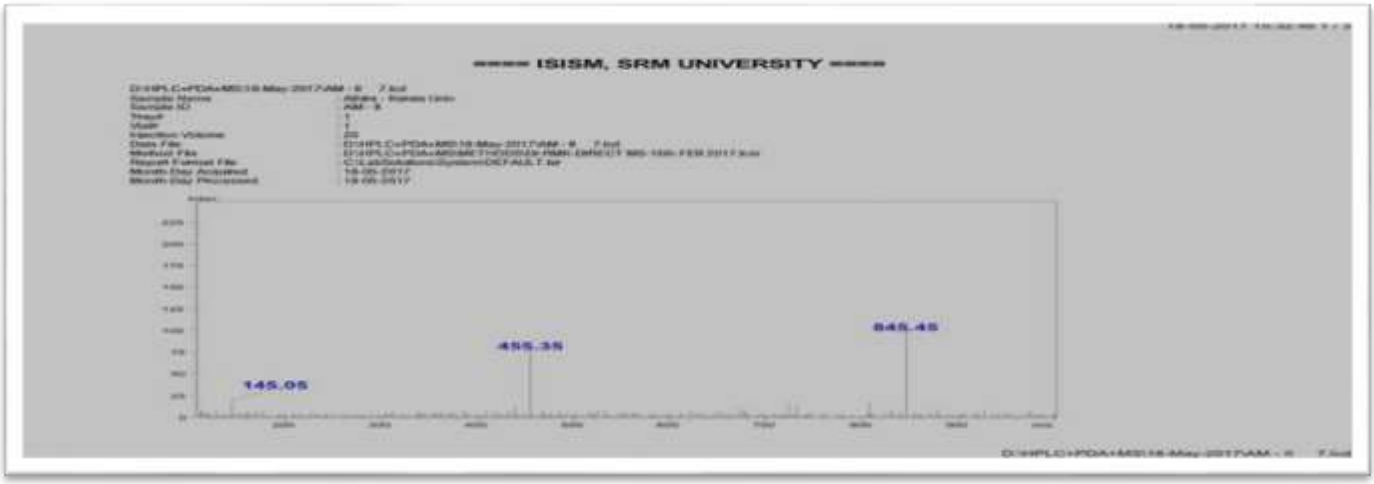

Figure 2: LC–MS spectra of the isolate

MASS spectroscopy

The configuration of the *Apis mellifera* (L.) isolate was explained by using an LC-MSD TRAP-SL 2010 A-SHIMADZU instrument. The spectrum shows a base peak at 455.35 and a molecular ion peak [M+] at 866.12 (Figure. 2). The IR, NMR, and LC–MS spectra revealed that the molecular weight of the isolated constituent was 866.74, which was confirmed to be procyanidin, a trimer of catechins (Figure.3).

Pharmacological studies

Acute oral toxicity studies

The isolated component was given orally by mixing with CMC and monitored for a period of 14 days. At 2000 mg/kg of the *Apis mellifera* (L.) isolate, there was no mortality or noticeable changes in weight or behavior, indicating the safety of the isolated chemical up to that dosage.

In vivo study

Compared with the control indomethacin, the individual compound of *Apis mellifera* (L.) at 400 mg/kg elicited a greater decrease in the rat paw edema model using carrageenan (Table 1). The absence of mortality or behavioral changes at 2000 mg/kg indicates the non-toxic nature of the *Apis mellifera* (L.) isolate. In the carrageenan-induced paw edema model, the isolate at 400 mg/kg showed significant anti-inflammatory activity, producing a greater reduction in paw edema compared to indomethacin, suggesting its potential to inhibit acute inflammation effectively.

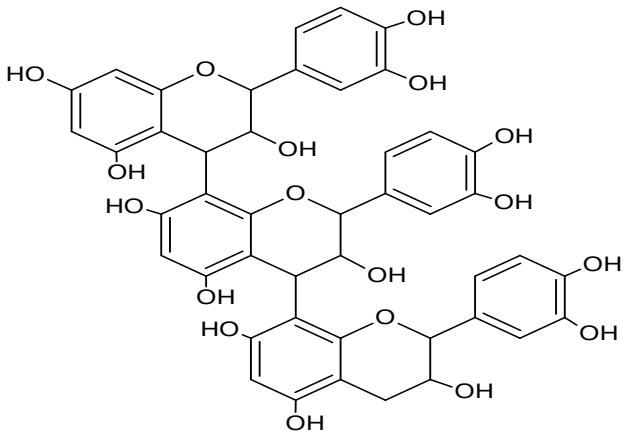

Figure 3: Procyanidins (Trimers of catechins)

Cotton pellet-induced inflammatory granuloma

At a dose of 200 mg/kg of the isolated compound, the % inhibition of the granuloma of wet cotton was 24.80%, and at a dose of 400 mg/kg, it was 43.8%. The percent inhibition of granulomas by the samples at 200 mg/kg and 400 mg/kg was 65.95 and 46.47, respectively (Table 2). For the standard drug at a concentration of 10 mg/kg, the % inhibition of the granulomas of wet and dry cotton was 53.21 and

58.29%, respectively. Compared with the standard, the isolated moiety at a dose of 400 mg/kg has anti-inflammatory activity similar to that of 200 mg/kg.

In the cotton pellet–induced granuloma model, the *Apis mellifera* (L.) isolate at 400 mg/kg produced a significant reduction in granuloma weight compared to the control group. This indicates strong inhibition of the proliferative phase of inflammation, likely through suppression of fibroblast proliferation and collagen formation. The effect was comparable to that of indomethacin, suggesting that the isolate may act via similar anti-inflammatory pathways, possibly involving cyclooxygenase inhibition or modulation of inflammatory mediators.

Table 1: Anti-inflammatory effects of the isolate on carrageenan-induced paw edema

Groups	Dose	15 min	30 min	60 min	120 min	180 min
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Control	0.1 ml/kg	1.48±0.08	1.51±0.09	1.27±0.50	1.27±0.03	1.31±0.15
Standard	10 mg/kg	1.47±0.08	1.23±0.04**	0.60±0.04***	0.30±0.05***	0.14±0.02***
Test drug	200 mg/kg	1.36±0.05	1.29±0.03*	1.13±0.02**	1.07±0.03**	0.72±0.02**
Test drug	400 mg/kg	1.30±0.09	1.17±0.04**	0.99±0.03***	0.82±0.02***	0.62±0.07***

The data are expressed as the means ± S.E.M.s, n = 6. The experimental groups were compared via Tukey's test after one-way ANOVA. ns-nonsignificant; values differ considerably from those of the control group; **P<0.01; ***P<0.001; *P<0.05

Table 2: Anti-inflammatory activity of isolates produced from cotton pellet-induced granulomas

Groups	Dose	Cotton's Wet weight	Cotton's Dry weight	% of inhibition
		Mean±SEM	Mean±SEM	
Control	0.01 ml	111.01±0.82	39.63±0.54	–
Standard	10 mg/kg	51.95±0.81***	16.52±0.30***	68.00
Test drug	200 mg/kg	83.51±0.72**	28.43±0.34**	65.95
Test drug	400 mg/kg	63.35±0.52***	21.21±0.44***	46.47

The data are expressed as the means ± S.E.M.s, n = 6. The experimental groups were compared via Tukey's test after one-way ANOVA. ns-nonsignificant; values differ considerably from those of the control group; **P<0.01; ***P<0.001; *P<0.05

Conclusion

This study identified and characterized a bioactive flavonoid, procyanidin, from *Apis mellifera* through chromatographic and spectroscopic techniques. The findings establish *Apis mellifera* as a promising source of naturally derived anti-inflammatory compounds and contribute to the growing evidence supporting the pharmacological relevance of bee-derived constituents. The isolate's safety profile and potential anti-inflammatory mechanism underscore its value as a lead candidate for further drug development. Future research should focus on elucidating the precise molecular mechanisms underlying the anti-inflammatory effects of procyanidin, including its interaction with inflammatory mediators and signalling pathways. In-depth pharmacokinetic, toxicological, and formulation studies, followed by preclinical and clinical evaluations are warranted to translate this compound into a viable therapeutic agent. Integrating modern analytical and computational approaches may further enhance understanding of its bioactivity and therapeutic potential.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Torto B, Carroll MJ, Duehl A, Fombong AT, Katzav-Gozansky T, Nazzi F, Soroker V, Teal PEA. Standard methods for chemical ecology research on *Apis mellifera*. J Apic Res. 2016;52(4):220-38.
2. Schmitt T, Herzner G, Weckerle B, Schreier P, Strohm E. Volatiles of foraging honey bees (*Apis mellifera*) and their potential role as semiochemicals. Apidologie. 2007;38(2):164-70.
3. Slessor KN, Kaminski LA, Winston ML. Semiochemical basis of the retinue response to queen honeybees. Nature. 1988;332(6161):354-6.
4. Matysiak J, Swiatly A, Hajduk J, Matysiak J, Kokot ZJ. Influence of honeybee sting on peptidome profile in human serum. Toxins. 2015;7(3):1808-20.
5. Lee PC, Winston ML. Effect of reproductive timing and colony size on survival, offspring colony size, and drone production in the honey bee (*Apis mellifera* L.). Ecol Entomol. 1987;12(3):187-95.
6. Slessor KN, Winston ML, Le Conte Y. Pheromone communication in the honeybee (*Apis mellifera* L.). J Chem Ecol. 2005;31(11):2731-45.

7. Keeling CI, Slessor KN, Higo HA, Winston ML. New components of honeybee (*Apis mellifera* L.) queen retinue pheromone. Insect Soc. 2003;50(2):150-5.
8. Racey PA, Filmer RS. Effect of the size of honey bee colonies on food consumption, brood rearing, and the longevity of the bees during winter. J Apic Res. 1968;7(2):103-12.
9. Findlay R, Eborn B, Jones W. Effect of colony creation method and beekeeper education on honey bee (*Apis mellifera*) mortality. J Ext. 2015;54(1):41-5.
10. Ochoa ML, Guerrero A, Peña J, Rojas A, Rutiaga JG. Spectroscopic study of honey from *Apis mellifera* from different regions in Mexico. Spectrochim Acta A Mol Biomol Spectrosc. 2017;173:972-8. doi:10.1016/j.saa.2016.10.058
11. Owayss AA, Rady MM, Gadallah FM. Pigmentation of some honey bee (*Apis mellifera* L.) products. Fayoum J Agric Res Dev. 2004;18(2):121-32.
12. Nugraheni ZV, Zetra Y, Trianita AM, Syahputra MY, Firmany AR. Antioxidant activity in natural beehive's (*Apis mellifera*) bioactive compound from Malang, Indonesia. AIP Conf Proc. 2016;1755: 080003.
13. Selvan KA, Prabhu T. Extraction of propolis from beehives and characterization of its constituents and medicinal properties: a review. Int J Adv Eng Technol. 2010;1(1):50-3.
14. Ampe C, Jacobs F, Fleming M. Isolation and characterization of abaecin, a major antibacterial response peptide in the honey bee (*Apis mellifera*). Eur J Biochem. 1990;187(2):381-6.